Chk1 Activation and the Nuclear/Cytoplasmic Ratio

ATR and Chk1 are important components of a cell cycle checkpoint pathway. In this issue of *Developmental Cell*, Conn et al. shed a novel light on the molecular mechanism of Chk1 activation and raise the possibility of a developmental checkpoint that regulates Chk1 in response to the nuclear/cytoplasmic ratio.

ATR and Chk1 are important components of a cell cycle checkpoint pathway that senses damaged or incompletely replicated DNA to inhibit entry into mitosis, which is controlled by cyclin-dependent kinases (Cdks). Wee kinase negatively regulates Cdk activity by phosphorylation on Tyr-15, and this inhibitory phosphorylation is removed by the Cdc25 family of phosphatases. Chk1 inhibits mitosis by phosphorylating Cdc25A and Cdc25C, resulting in their ubiquitination and degradation or sequestration by the 14-3-3 family of proteins. Chk1 level is constant, but it is activated by ATR, which in turn is activated by damaged or incompletely replicated DNA.

Interestingly, while the Chk1 cell cycle checkpoint pathway can be activated in vitro in *Xenopus* egg extracts by addition of damaged DNA or by blocking DNA synthesis, neither blocking DNA synthesis nor DNA damage can arrest cell cycles of the intact *Xenopus* embryo prior to the midblastula transition (MBT) (Anderson et al., 1997; Newport and Dasso, 1989). The MBT is a special period in embryonic development with significant changes in cell cycle regulation and in gene expression. Early embryonic development generally starts with rapid cell divisions that consist of alternating S and M phases driven by maternally derived products, and it exhibits little zygotic transcription. At the MBT, the gap phase of the cell cycle is first introduced, and widespread zygotic transcription initiates. The inability of pre-MBT embryos to activate cell cycle checkpoint pathways in response to DNA damage or replication blocks could be due to the presence of a maternal inhibiting factor, the absence of zygotic factors required for the cell cycle checkpoint response, or the inability of damaged DNA to send a sufficiently strong signal at low nuclear/cytoplasmic (N/C) ratio. As cell cycle checkpoints can be activated in *Xenopus* egg extracts by addition of DNA, it is likely that all the factors required for the cell cycle checkpoint response are present in the early embryos. Therefore, the N/C ratio could be an important factor that is lacking in the early embryos, resulting in their inability to activate the Chk1 cell cycle checkpoint. In fact, the N/C ratio has been implicated in controlling the onset of cell cycle slowing and arrest in *Drosophila* (Edgar et al., 1986; Sibon et al., 1997).

Models for the activation of Chk1 at the MBT generally suggest that as the N/C increases, the increased DNA titrates out a maternal replication factor, resulting in slowed or incomplete DNA replication, which results in ATR-dependent activation of Chk1 and inhibits entry into mitosis. In this issue of *Developmental Cell*, Conn et al. (2004) investigate DNA damage-induced signaling through Chk1 in early *Xenopus* development, and they present intriguing findings that suggest a new developmental mechanism for the activation of the Chk1 cell cycle checkpoint pathway.

By injecting oligonucleotides into early embryos, Conn et al. show that high quantities of double-stranded DNA are sufficient to slow early cleavage cycles, to induce Chk1 activation, and to inhibit Cdk activity. This activation results in the inhibition of Cdc25A and Cdc25C, leading to phosphorylated (inactive) Cdk, and can be inhibited by caffeine, a known inhibitor of the ATM/ATR family. Therefore, early embryos can elicit a checkpoint response when given sufficient amount of DNA or free DNA ends. Surprisingly, by using different lengths of oligonucleotides, Conn et al. show a dose-dependent relationship in which the activation of Chk1 is dependent on the total amount of DNA injected rather than on the total number of free DNA ends. In fact, linearized plasmid can also induce Chk1 activation even at a concentration of about 100-fold lower free DNA ends. Even more surprising is the observation that injection of a large amount of circular plasmid (approximately equal to the amount of total DNA at the MBT) on its own is sufficient to induce activation of Chk1. Because this intact plasmid would not be expected to activate the DNA damage pathway, and because activation of Chk1 occurs much earlier than the replication of the plasmid DNA would be expected to have occurred, these observations suggest a potential novel mechanism by which Chk1 can be activated. As shown in Figure 1, it is possible that Chk1 could be activated directly when the N/C ratio reaches a certain threshold in addition to being activated in response to damaged or unreplicated DNA.

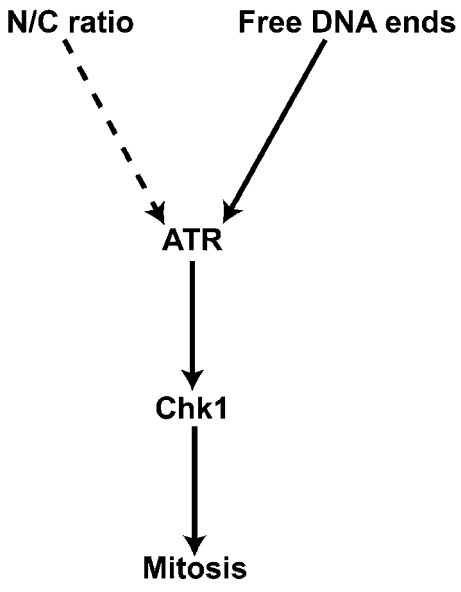

Figure 1. The ATR and Chk1 Pathway Is Regulated Both by the Free DNA Ends and by the Nuclear/Cytoplasmic Ratio

Furthermore, the pathway that detects free DNA ends and the pathway that detects the N/C ratio appear to be able to activate Chk1 synergistically (Conn et al., 2004).

Such a model of Chk1 activation will have significant implications for our understanding of the regulation of cell cycles at the MBT. In *Xenopus*, the MBT occurs after the 12th cell cycle, and developmental slowing of the cell cycle was shown to be dependent on the activity of Chk1 (Carter and Sible, 2003; Shimuta et al., 2002). Similarly in *Drosophila*, the homologs of ATR (*mei-41*) and Chk1 (*grapes*) are required to terminate the cleavage cell cycle at the MBT (Sibon et al., 1999, 1997). The results by Conn et al. raise the possibility that, at the MBT, the ATR/Chk1 pathway is activated in response to a predetermined N/C ratio (a developmental checkpoint) or to a large amount of free DNA ends (a cell cycle checkpoint), or more likely in response to both of these two signals. Clearly, additional studies will be needed to distinguish these possibilities. Furthermore, questions about the mechanism that is responsible for sensing the N/C ratio and the mechanism by which the N/C ratio is relayed to the regulation of ATR or Chk1 remain to be addressed. In this regard, it is of interest to note that the expression of *frühstart* (*frs*), a gene reported to be important for cell cycle arrest in early *Drosophila* development, is apparently also controlled by the N/C ratio (Grosshans et al., 2003). In haploid embryos, frs expression is delayed by one cell cycle. Nothing is known about the molecular function or regulation of this protein, but it is possible that the N/C sensor that activates *frs* expression could also be linked to Chk1 activation.

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Chewing the Fat: Regulating Autophagy in *Drosophila*

Autophagy is the major cellular process responsible for bulk cytoplasmic degradation. Two reports in this issue of *Developmental Cell* describe how both PI3 kinase and TOR signaling in *Drosophila* are critical for controlling autophagy in response to developmental and environmental cues.

Autophagy is the major mechanism by which organelles and long-lived proteins are degraded in eukaryotes. In this process, portions of the cytoplasm are sequestered in double membrane vesicles known as autophagosomes. These subsequently fuse with lysosomes, resulting in degradation of their cytoplasmic contents. Studies in yeast have yielded valuable insights into the control of autophagy (Levine and Klionsky, 2004). These single cells exhibit a simple lifestyle, growing when environmental nutrients are abundant and ceasing growth when nutrients become limiting. Autophagy is rapidly induced by nutrient starvation in yeast, providing a critical means for recycling nonessential macromolecules to sustain viability under suboptimal conditions. The conserved nutrient-responsive TOR signaling pathway appears to be the chief regulator of autophagy in this context. Moreover, key genetic studies in yeast have begun to unravel the molecular machinery of autophagy, contributing a framework for understanding how this process is regulated (Klionsky et al., 2003).

The induction of autophagy following nutrient starvation has also been well documented in mammals, both in cell culture and, recently, in vivo, using transgenic mice that express a fluorescent marker for autophagosomes (Levine and Klionsky, 2004). Multicellular organisms have also evolved to use autophagy to control other critical events such as cellular remodeling during development, programmed cell death, and the turnover of damaged organelles (Klionsky et al., 2003). Indeed, aberrant autophagy has been implicated in aging and cancer. Despite this, few studies have genetically analyzed the regulation and role of autophagy in metazoans. Two studies reported in this issue begin to tackle this topic using the power of *Drosophila* genetics.

The larval period in *Drosophila* is characterized by a tremendous increase in mass with growth occurring predominantly in larval-specific tissues such as the salivary gland, gut, epidermis, musculature, and fat body. This growth is driven by nutrition-dependent activation of the insulin/PI3K and TOR signaling pathways. Upon removal of dietary protein, these pathways are inactivated and growth is shut down (Britton and Edgar, 1998; Britton et al., 2002). By analyzing the larval fat body, both Scott et al. (2004) and Rusten et al. (2004) demonstrated that this cessation of growth is accompanied by marked increases in autophagy. Using a combination of electron and fluorescent microscopy, they observed